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Annual Report for Award Number DAMD17-01-1-0787

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Below is the Annual Report for the study entitled "Use of DNA microarrys to identify diagnostic signature transcriptional profiles for host responses to infectious agents".

Restatement of specific aims

There are five main aims that are relevant to all agents under initial investigation, *Bacillus anthracis, Burkholderia mallei, Francisella tularensis,* multi-drug resistant *Mycobacterium tuberculosis* and *Yersinia pestis*:

1. Develop human and mouse DNA chips to study transcriptional activation and repression by pathogens.

2. Develop appropriate *in vitro* models to explore the interactions of host cells with the pathogen and its toxins/constituents.

3. Characterize the pattern of genes activated or repressed:
   a. by infection with virulent strains vs. attenuated organisms/mutants as an approach to ensure specificity
   b. in infected cells from vaccinated vs. unvaccinated healthy individuals

4. Using the mouse as an *in vivo* model of human infection, characterize changes in gene expression following infection with virulent and avirulent organisms.

5. Based on the above findings, develop DNA chips and assays for associated disease markers that focus on genes and their products that provide the best discrimination among these agents. Apply these DNA chips and assays for disease markers to other bio-terrorism agents as well as other common infectious diseases to confirm the specificity of the diagnostic approach.

Preliminary results.
Aim 1. Microarrays
Spotted arrays
The human microarray was prepared by a set of arraying 65-mer oligonucleotides purchased from Compugen (Jamesburg, NJ), representing predicted genes. A total of 19,200 spots which include 200 glyceraldehyde-3-phosphate dehydrogenase (GAPDH

controls) and 288 buffer spots (negative controls) were robotically spotted using GeneMachine's Omni Grid microarrayer on poly-L-lysine-coated slides and blocked by the succinic anhydride-sodium borate-1methyl-2-pyrrolidinone method.

Standardization of cDNA Labeling

The microarray chips described above were standardized using commercially available total liver and kidney RNA from normal human subjects (Stratagene, CA). The RNA labeling was performed using cDNA submicro array kit Cy3 A100731V12, Cy5 A100741V12 (Genisphere, Hatfield, PA) using a two step labeling protocol. Briefly, total liver and kidney RNA (5.0 μg) and 5 pmol of Cy3 and Cy5 capture sequence primers were brought to 11 μl with DEPC-treated water and incubated for 10 min at 80^0 C. At 42^0C an equal volume of reaction mix containing 10 mM dNTPs, 5 μl of 5X first strand buffer (Invitrogen, CA), 2μl of 0.1M DTT, 1μl of Superasin RNase inhibitor (Genisphere, PA) and 200 U Superscript II reverse transcriptase (InVitrogen, CA) was added, and then reaction was incubated 2 h at 42^0C. The reaction was terminated by adding 3.5 μl of 3.5ul 0.5M NaOH/50mM EDTA and by incubating it at 65°C for 10 min. The reaction was neutralized by adding 5μl 1M Tris-HCl, pH 7.5. The Cy3 and Cy5 reactions were then combined and volume was adjusted to 70 μl by adding 1 M TE buffer, pH 8.0. The human chips were standardized by reciprocal labeling of liver and kidney RNA and analysis of reciprocal gene expression (data not shown).

Mouse arrays

Affymetrix Murine Genome U74Av2 arrays were used for analysis of mouse genes induced by *F. tularensis* (LVS) infection. The Murine Genome U74v2 Set, consisting of three GeneChip® probe arrays, contains probe sets interrogating approximately 36,000 full-length mouse genes and EST clusters from the UniGene database (Build 74).

Algorithm development

Last year, we described our development of the Directional Change Assessment Algorithm as a key data-mining tool for this project. Since our last report we have incorporated two significant improvements upstream of this algorithm, Lowess Normalization and Noise filtering, to provide more efficient and reliable data analysis.

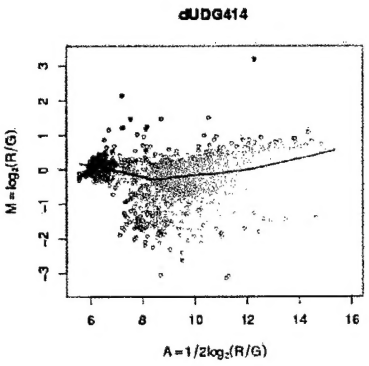
Lowess Normalization

This method helps us normalize the fluorescent intensity of one chip to another before we compare the data. First we transfer the R (red channel intensity) and G (green channel intensity) values to (M, A) values. The transfer formulas are: $M_{original} = \log_2 R - \log_2 G$ and $A = (\log_2 R + \log_2 G)/2$. Second, we make a scatter plot of $M_{original}$ vs. A and construct a LOWESS curve (locally weighted scatter-plot smoother) of that scatter plot. The values on the curve are M_{lowess}. Subtract the M_{lowess} from $M_{original}$ value, the residues are our normalized M values. $M_{normalized} = M_{original} - M_{lowess}$ .Examples of Lowess normalization are shown below.

No Normalization

Non-normalized data $\{(M,A)\}_{n=1..5184}$:

$M = \log_2(R/G)$

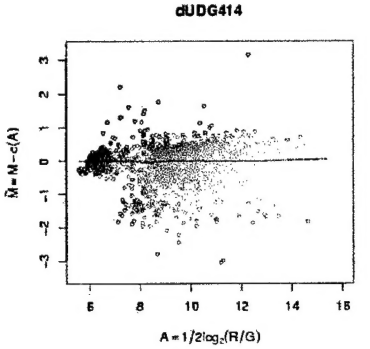


Global (lowess) Normalization

Global normalized data $\{(M,A)\}_{n=1..5184}$:

$M_{norm} = M - c(A)$

where $c(A)$ is an *intensity dependent* function.

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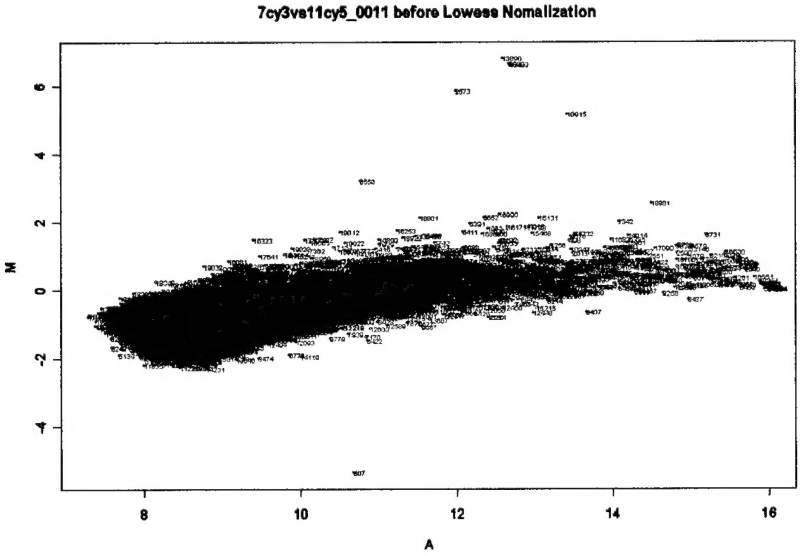
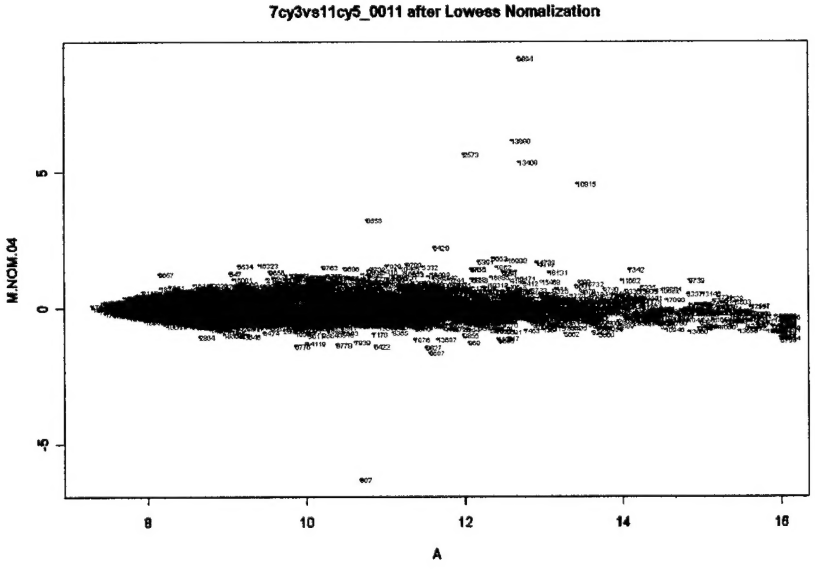




Noise modeling
There are different sources of noise in microarray experiments. We cannot correct for the biological noise but we can take it into account in the analysis with statistics. The noise due to the technique is reproducible and can be modeled and accounted for. There is a greater variance of the fold changes at low signal intensities. The whole purpose of the error model was to eliminate the non-trustworthy fold changes from the analysis. In order to model the noise we wanted to draw the boundary of the fold changes for each replicate. We binned the genes according to their average expression value (bin size of 200) for each pair of replicates. We then took the 95% percentile of the fold changes of each bin and performed a linear regression against the inverse of the average intensity value of the bin. For all the replicates and each image analysis methods we recorded the slope value and intercept. The slope gives an indication of the noise level in the low end whereas the intercept gives an indication of the noise level for high average intensities.

Aim 2. *in vivo* and *in vitro* infections

Agents:
Bacillus anthracis:
V1B: vollum 1B, contains both virulence plasmids, pXO1 and pXO2
Sterne: lacking the pXO2 plasmid for capsule production.
Burkholderia mallei:
China 7: virulent strain, also known as ATCC 23344
NVSL 85-503- clinical isolate- avirulent strain
Francisella tularensis:
LVS: live vaccine strain
Mycobacterium tuberculosis:
H37Ra: avirulent laboratory strain
MDR 307: clinical isolate, resistant to INH, rifampin, streptomycin and ethambutol. This strain is susceptible to pyrazinamide.
Yersinia pestis:
CO92: virulent, contains all virulence plasmids and factors
CO92 pgm-lcr-: avirulent strain lacking the low calcium response plasmid which contains the yops genes (virulence genes) and the plasmid coding for plasminogen. (?)

Growth conditions for the agents
In preparation for the infections for these experiments, each of the agents was stocked in 50% glycerol and stored at –80C for future use. *Bacillus anthracis* and *Yersinia pestis* were grown overnight in Brain Heart Infusion Broth at 37C to an OD600 of approx 0.6. *Burkholderia mallei* was inoculated into LB broth + 4% glycerol and incubated at 37C until it reached an OD600 of 0.6, which occurred after approximately 5 days. *Francisella tularensis* was inoculated onto Mueller Hinton agar plates with additives and grown at 37C with CO_2 for 5 days until colony formation was seen. Colonies were then suspended in glycerol. *Mycobacterium tuberculosis* is grown in Bactec 13A Mycobacterium medium.

After DNA and/or RNA is isolated from the agent or from cells infected with the agent, part of the nucleic acid sample is plated onto nutrient agar to test for sterility. NBY agar is used for *B. anthracis*, EMB agar for *Y. pestis*, blood agar for *B. mallei*, 7H11 agar for *M. tb* and modified MH for *F. tularensis*.

Media:

NBY medium	**Mueller Hinton medium**	**Bactec 13A Mycobacterium medium:**
8g/L nutrient broth	MH broth base 21g/L	7H9 broth base: 0.47% w/v
3g/L yeast extract	NaCl 5g/L	Casein hydrolysate: 0.1% w/v
15g/L agar	Proteose peptone/tryptone: 10g/L	Na polyanetholesulfonate 0.025% w/v
	Bacto agar: 16g/L	Polysorbate 80: 0.02% w/v
	10% glucose 10ml/L	Catalase: 1440 units
	2.5% ferric pyrophosphate 10ml/L	bovine serum albumin: 15% w/v
	Isovitale X (cysteine) 20ml/	
	Fetal bovine serum 25ml/L	

Infection conditions for agents

All agents are or will be grown to an OD 600 of 0.6 for infection while shaking and the blood or cell-line will be infected with an MOI of 1 bacterium: 1 monocyte. All infections will occur with mid-log phase vegetative cells with the exception of *M. tb* and *B. anthracis*. We consider the spore of *B. anthracis* as the infectious particle and thus we have produced spores to be used for the infections. The MOI for the *B. anthracis* infections is also 1:1. For M.tb, cultures are grown up to a Growth Index of 300- 400, which is OD600 of approximately 0.3-0.5, with BSA at 1%. One ml aliquots are made and frozen for further use in infections. With one aliquot we also make a standard curve (using 12B 4 ml bactec bottles, which have the same composition as the 13A but without Catalase, SPS, polysorbate and enrichment media) to estimate the amount of mycobacteria per ml. We use this figure to calculate the amount of bacterial suspension to be used in the infection.

***B. anthracis* pore production protocol**

Bacteria are grown in Brain Heart Infusion broth at 37C until cells reach $1x10^9$cells/ml. The bacteria are then streaked onto NBY plates and incubated at 37C for 7days. The colonies are scraped off the plates and added to 10ml of sterile dH20 to produce a spore suspension. This spore suspension is incubated at 65C in a water bath for 30 minutes to kill any remaining vegetative cells. The spores are then centrifuged at 7000 rpm for 15minutes and resuspended in water. Stocks are stored in 50% glycerol at 4C. Dilutions of the suspension are made and spread onto NBY plates to determine the concentration of the spore stock. Before infection, an aliquot of the spore suspension is spun down at 5000rpm for 10minutes and the spores are then resuspended in RPMI w/ L-glutamine.

Whole Blood Model

Blood is obtained from healthy human volunteers the morning of infection, as per approved Human Use Protocol. We draw 240ml that is used for two parallel experiments: infection for total RNA from whole blood, and infection for total RNA from the $CD14^+$

monocyte subpopulation. The blood sampled are infected with the virulent strain, the avirulent strain and a mock infection. We use 20ml of blood per condition. Two time points are used: 3 and 8 hours. With *M. tb*, we include an additional time point of 24 hours, in view of its extremely slow growth rate. At each time point the samples are processed in two ways. For the samples from which we will isolate RNA from the WB cell population, the RBCs are lysed and Buffer RLT is added to preserve the RNA for the isolation procedure. The cell lysate is placed at –80 until RNA isolation occurs. For the samples designated for RNA from the monocyte population, we perform a Ficoll extraction of the PBMC layer. We then wash the PBMCs, label them with $CD14^+$ magnetic beads and pass them through an automated cell separator (Miltenyi AutoMacs). The cell fractions, both the positive (CD14+) and negative (non-labelled) are then centrifuged and resuspended in Buffer RLT with B-mercaptoethanol to preserve for RNA extraction.

Infections to date (whole blood model):
We have performed two TB infections and seven anthrax infections. Two of the anthrax infections cannot be used because the blood clotted severely in one case, and in the other the blood turned black at later time points, presumably because of lysis by both virulent and avirulent *B. anthracis* infection. We performed a growth curve in blood, which was a third experiment (see section on the killing curve experiment, below) and from these data excluded the 24 hr time point for *B. anthracis* infection. One of the anthrax infections and the two TB infections yielded preliminary data but since RNA yields were lower than expected, we shifted our experimental design towards the whole model and added the THP1 infection. We have successfully performed three anthrax infections, have made and are continuing to make RNA in the desired quantity for microarrays. The CAG is processing these RNA samples as this report is prepared.

THP1 human monocyte cell line
The rational for including THP1 infections in our protocol is the following: it is difficult to extract enough RNA from CD14+ cells after infection of whole blood successfully and consistently to hybridize 19,200+ genes on the spotted chips described above; 5 ugm is required. We chose to isolate total RNA from whole blood, which gives consistently high yields. We propose that if we find specific markers among total blood cell RNAs that may serve as markers to differentiate one infection from another, we can study RNA transcription in the human macrophage-like cell line THP1 to see whether CD14+ cells might be responsible for the expression of these candidate genes..

THP1 cells are grown in RPMI with 10%FBS and 2mM L-glutamine at 37C in 5% CO_2 until they reach a minimum concentration of 1.8×10^7 cells. PMA (polymyristate acetate) is added at a concentration of 20ng/ml to differentiate the cells. (Sly et al, 2001) Once the cells are differentiated and attached to substrate, they are infected with the avirulent and virulent strain of the agents being studied. There are also uninfected controls for each time point. Infection proceeds for 3 and 8 hours when the media is removed and Buffer RLT with B-mercaptoethanol is added to the monolayer, and RNA is isolated from the infected and uninfected macrophages. This RNA is then used for the microarrays. We have performed two THP1 infections with *Bacillus anthracis*; RNA production is in process.

RNA isolation

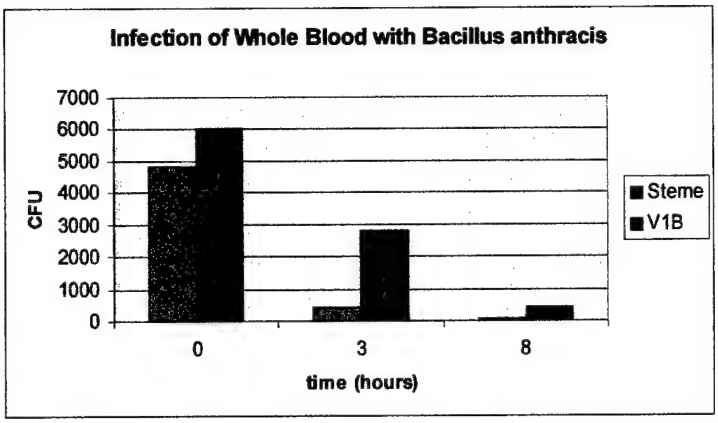
The Qiagen kits, mini or midi, are used for the first isolation of RNA. Then a phenol:chloroform extraction is performed with the use of phase lock gels to ensure sterilization of any remnant bacteria, there is an isopropanol precipitation followed by an ethanol wash. All RNA is dissolved in sterile DEPC water. The OD of the RNA is taken, as well as the 260/280 for purity. Reverse transcriptase PCR is then performed on the samples, to test for DNA contamination and to test for amplification of B-actin (acts as a control). RNA is then delivered to the Center for Applied Genomics for microarray analysis.

Sample		Condition	RNA(ng/ul)	total RNA(ug)	260/280
37#0206	WB	3uninfected	78	3.9	2.1
38#206	WB	3uninfected	3650	182.5	2.1
39#0206	WB	3Sterne	2078.3	103.9	2.2
40#0206	WB	3Sterne	157.1	7.855	2.1
41#0206	WB	3V1B	159.6	7.98	2.1
42#0206	WB	3V1B	147.5	7.375	1.9
43#0206	WB	8uninfected	115	5.75	2
44#0206	WB	8uninfected	1007.3	503.65	2.2
45#0206	WB	8Sterne	92.3	4.615	2.1
46#0206	WB	8Sterne	3120.9	156.045	2.1
47#0206	WB	8V1B	147	7.35	2.1
48#0206	WB	8V1B	95.1	4.755	2.1

Killing curve experiment

We measured the growth of *Bacillus anthracis* in whole blood to determine which time points we should use for our whole blood infection model. We infected whole blood as above with the virulent or avirulent strain at one of three different MOIs; 10 bacteria per 1 monocyte, 5 bacteria per 1 monocyte, and 1 bacterium per monocyte. Uninfected controls were also followed. The samples were processed at 0, 2, 6, 9, and 24 hours post infection. Cultures were incubated at 37C on a rotisserie for the specified amount of time. At each time point, the samples were spun down, the extra media removed, sterile water was added to lyse the cells. The cells were centrifuged again, the supernatant was removed and the pellet was resuspended in RPMI. The two supernatants and the pellet were plated for CFUs.

While performing this experiment we also observed the change in the color of the blood. Once we reached the 9 hour time point, all samples with MOI of 10:1 had changed to black, and the MOI 5:1 were very close to the black/red color. At 24 hours, all tubes were black. This color change is likely due to lysis of the red blood cells and oxidation of hemoglobin. Due to this observation we decided upon the 8 hour time point. We repeated the experiment using only a MOI of 1:1 at 0, 3 and 8 hours. The data are represented below.



Aim 3. DNA microarrays

Affymetrix:
Samples consisting of RNAs from control and LVS infected mice were submitted for transcriptional profile via the Affymetrix Genechip microarray platform. Fifteen micrograms of biotin-labeled cRNA were fragmented and hybridized overnight (~16 hours at 45° C) on Murine Genome U34Av2 arrays. Washing and staining (Streptavidin Phycoerythrin) was done using the Genechip Fluidics Station 400 (Affymetrix) using the EukGE-WS2v4 protocol. Images were acquired using the Affymetrix Genearray scanner. Results were analyzed using Affymetrix Microarray Suite 5.0 and Affymetrix Data Mining Tool 3.0.

Prior to analysis each data set was normalized using global scaling. A comparative analysis was conducted using the controls (baseline) and the infected mice. Several metrics were used in determining a significant increase and decrease in gene expression. They included the change call and the signal log ratio. Transcripts with increase ("I") and decrease ("D") calls and signal log ratios greater than two in magnitude were considered highly significant. Two-dimensional hierarchical clustering was performed on the subset of data and are included in the addendum to this report.

Human arrays
For infection experiments, total RNA (5 μg) was isolated from whole blood and CD^+ 14 monocytes infected either with *B.anthracis* [Sterne (avirulent), vollum (virulent) and uninfected control] or *M.tuberculosis* $H_{37}Ra$ (avirulent) , multi-drug resistant (MDR) virulent strain of *M.tuberculosis* $H_{37}Rv$ and uninfected control. Uninfected controls and avirulent RNAs were always labeled with Cy3 primer whereas, the RNA from blood or monocytes infected with virulent strains was labeled with Cy5 primers. The labeling was performed as described above for the standard liver and kidney samples.

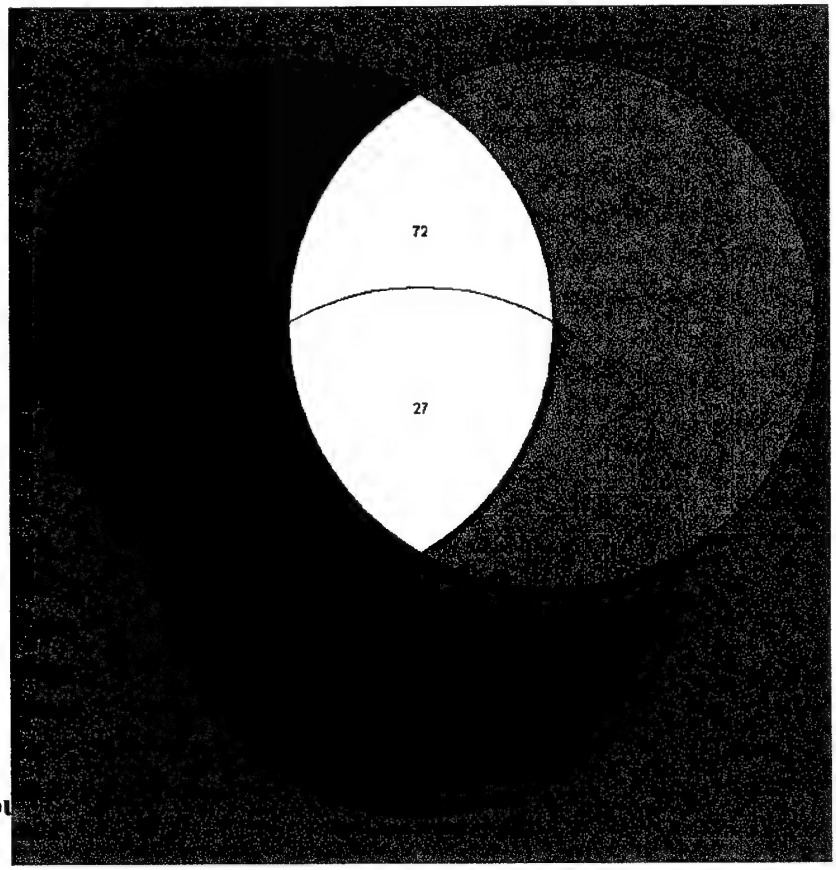
Hybridization of Microarrays
The hybridizations were performed using a Discovery automatic hybridization station from Ventana Medical System (Tucson, AZ). Typically, The microarray slides were bar coded using the manufactures instruction (Ventana, AZ) and then 200 μl of first hybridization mix containing 70 μl of labeled Cy3/Cy5 cDNA, 8 μl of 50X Denhardt's (Sigma, St. Louis, MO), 2 μl of Human cot-1 DNA, 20 μl of formamide (Sigma, St.

Louis, MO) and 100 µl of Ventana ChipHybe buffer was loaded on the arrays under the liquid cover slip. The hybridization was performed at 58^{0}C for 12 h. 201 µl of dendrimer mix was prepared for the second hybridization by adding 40µl formamide,73µl RNase free water, 73µl 20X SSC, 2µl 10% tween20, 8µl 50X Denhardt's, 2.5µl Cy3 dendrimer and 2.5µl Cy5 dendrimer and loaded on slides. The second hybridization was performed at 55^{0}C for 2 hours. After hybridization, slides were quickly washed twice by 1X reaction buffer ™ (Ventana), once with 2X SSC and then with 0.2XSSC respectively.

Scanning and Image Analysis: After hybridization and washing the microarray slides were scanned using a commercial laser scanner (GenePix4000, Axon Instruments Inc., Foster City, CA) with independent excitation of the fluorophores Cy3 and Cy5. The laser power was set to 100% and the PMT (photo multiplier tube) settings were changed depending upon the intensity of the array. The signal and background fluorescence intensities were calculated for each DNA spot using image analysis software (GenePixPro 4.0, Axon instruments) by averaging intensities of every pixel inside the target region.

Genes induced in human blood in response to infection with B. anthracis and MDR-TB.

Below is a Vein diagram comparing genes induced by infection of human whole blood with B. anthracis and MDR –TB. A table listing the identities of the genes and their induction ratios is found in Appendix 1.



Aim 4. Mou

Infection of Mice with *F. tularensis*

Female 5-6 week old BALB/c mice were employed. The vaccine strain *F. tularensis* LVS (ATCC 29684) was supplied by Dr. Karen Elkins, FDA. In the initial experiments, the bacteria will be grown at the FDA and the mice will be infected at that facility. Briefly, bacterial will be grown on modified Thayer-Martin agar. For intradermal and intraperitoneal inoculation 3.8 X 10^5 *F. tularensis* LVS was used. Tissues to be studied are PBL, spleen, lymph node, thorax; half will have RNA extracted and half will be snap frozen in liquid nitrogen in the OCT compound (Tissue Tek) and samples stored at -70 until sectioned. Data showing transcriptional profiles (below) are derived from spleen.

Genes induced in spleen cells from mice infected with *F.tularensis* LVS.

Preliminary results, shown in Appendix 3, suggest that in spleen cells from both ID and IP infected mice, as well as LPS treated, the major class of genes induced is related to immunoglobulin production. In addition, specific cytokine response to these infections has been measured and the data are under preparation (coordinated by Dr. Raveche).

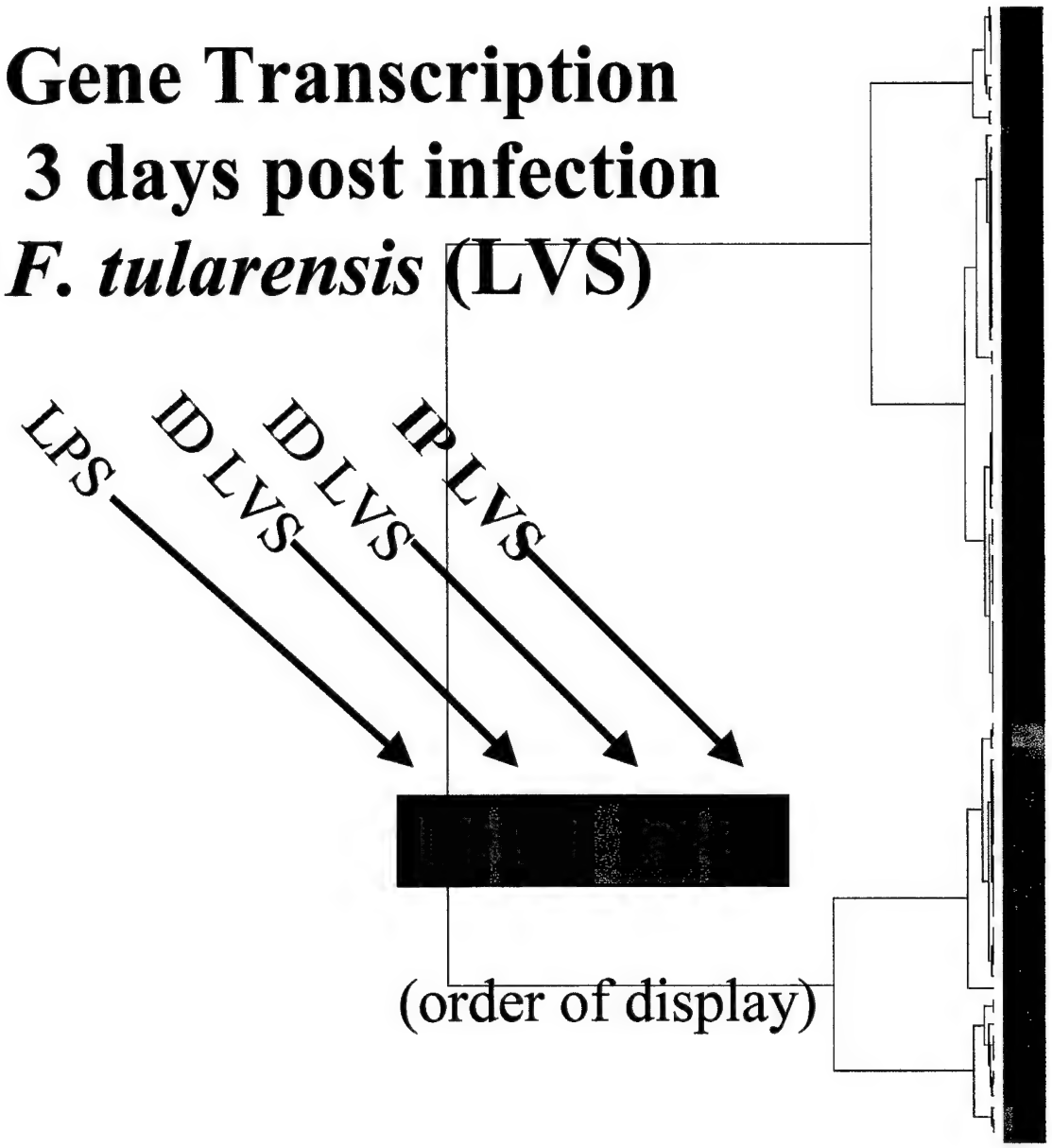
Conclusion.

Our preliminary data suggest that in both the mouse and human whole blood model, there may be genes whose expression forms a transcriptional profile that may be specific to the agent under study. However, the experiments are just now underway, and studies of more individuals will be required to confirm whether the markers identified so far are consistently expressed in the population in response to infection. In FY01, we will be extending our approach to include analysis of polymorphisms at the regulatory regions of various cytokines present in the individuals under study.

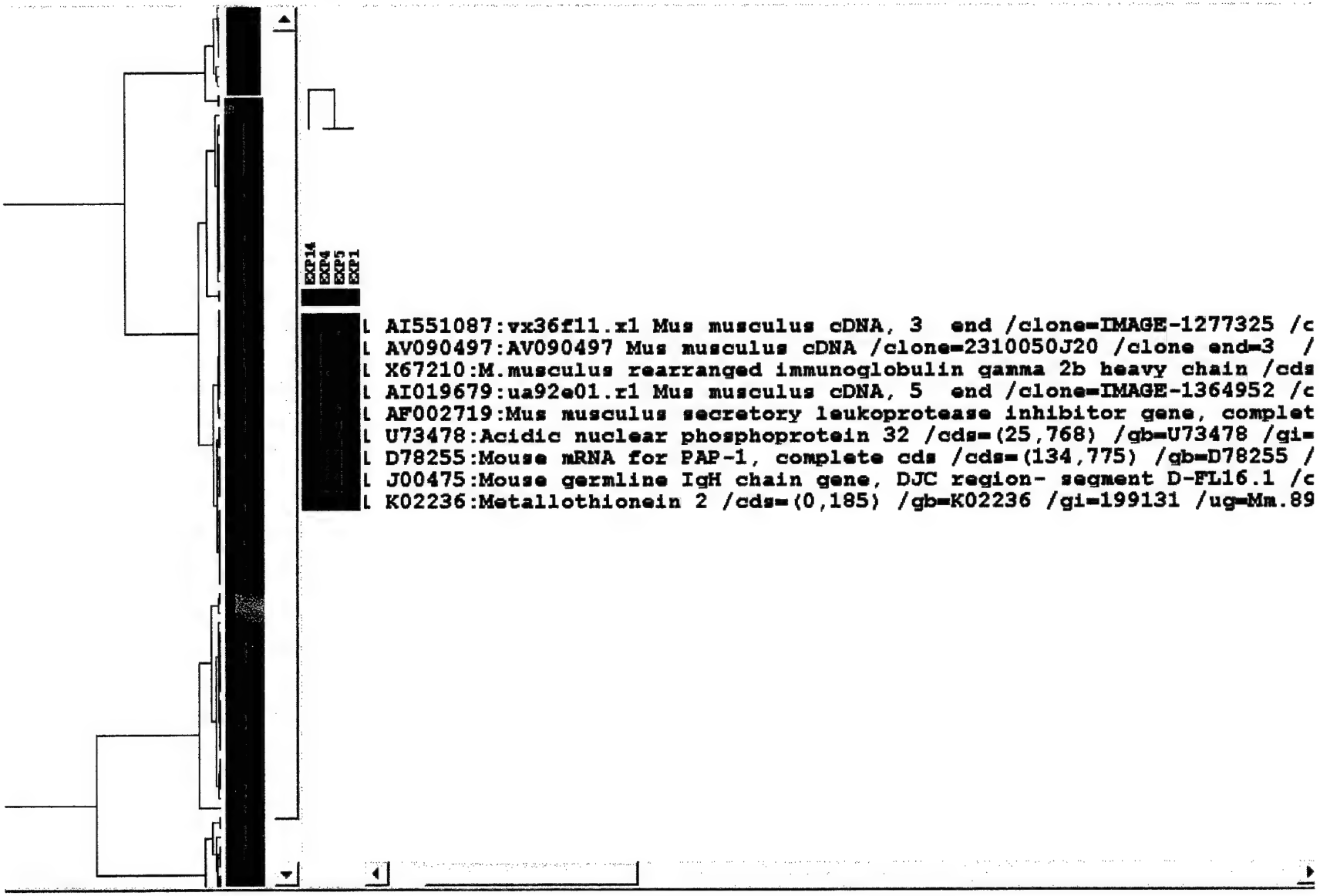
F. tularensis mouse infection

Sample number	Treatment/infection
1	IP
4	ID
5	ID
6	uninfected
13	untreated
14	LPS treated

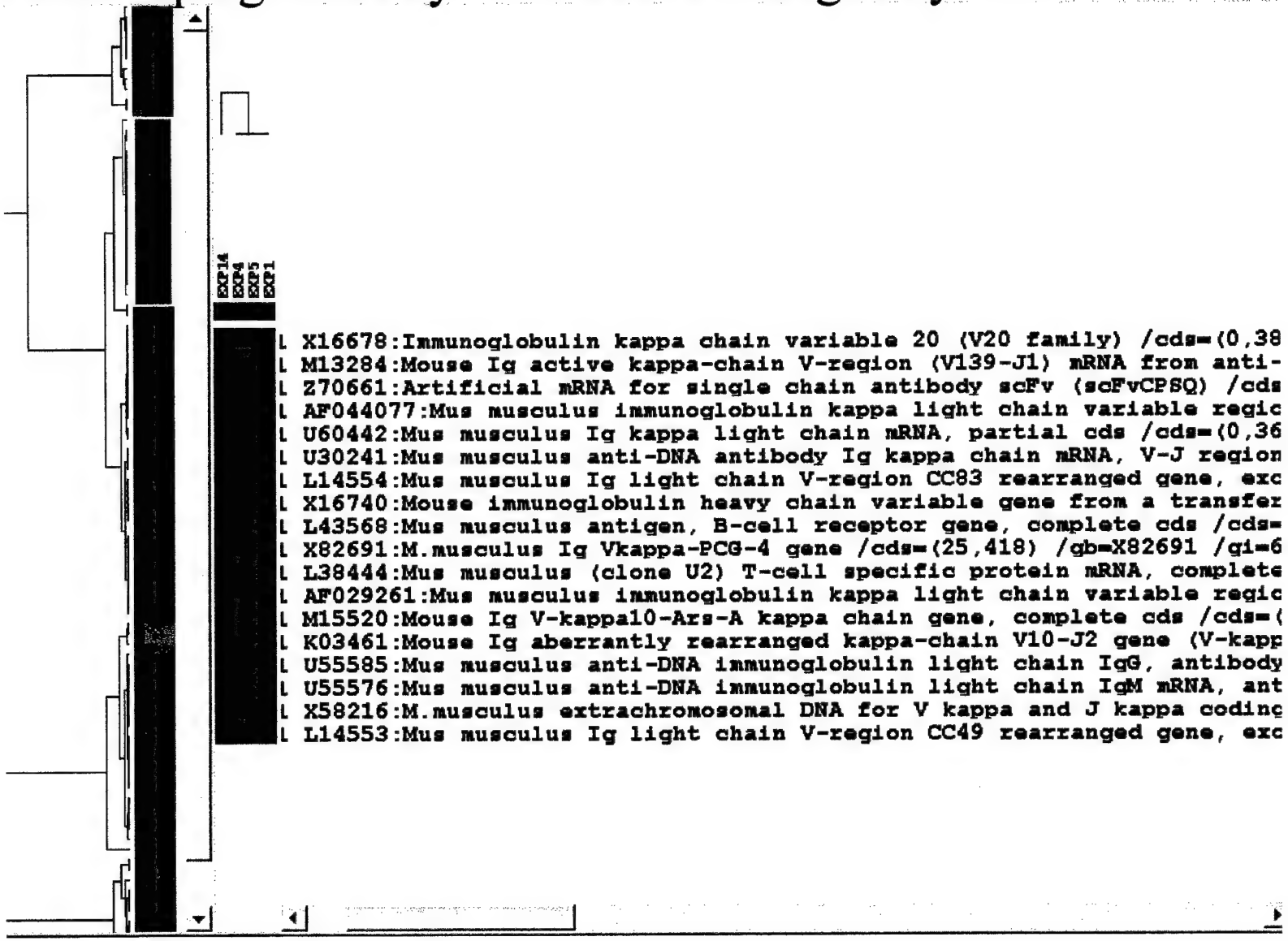
Gene Transcription
3 days post infection
F. tularensis (LVS)



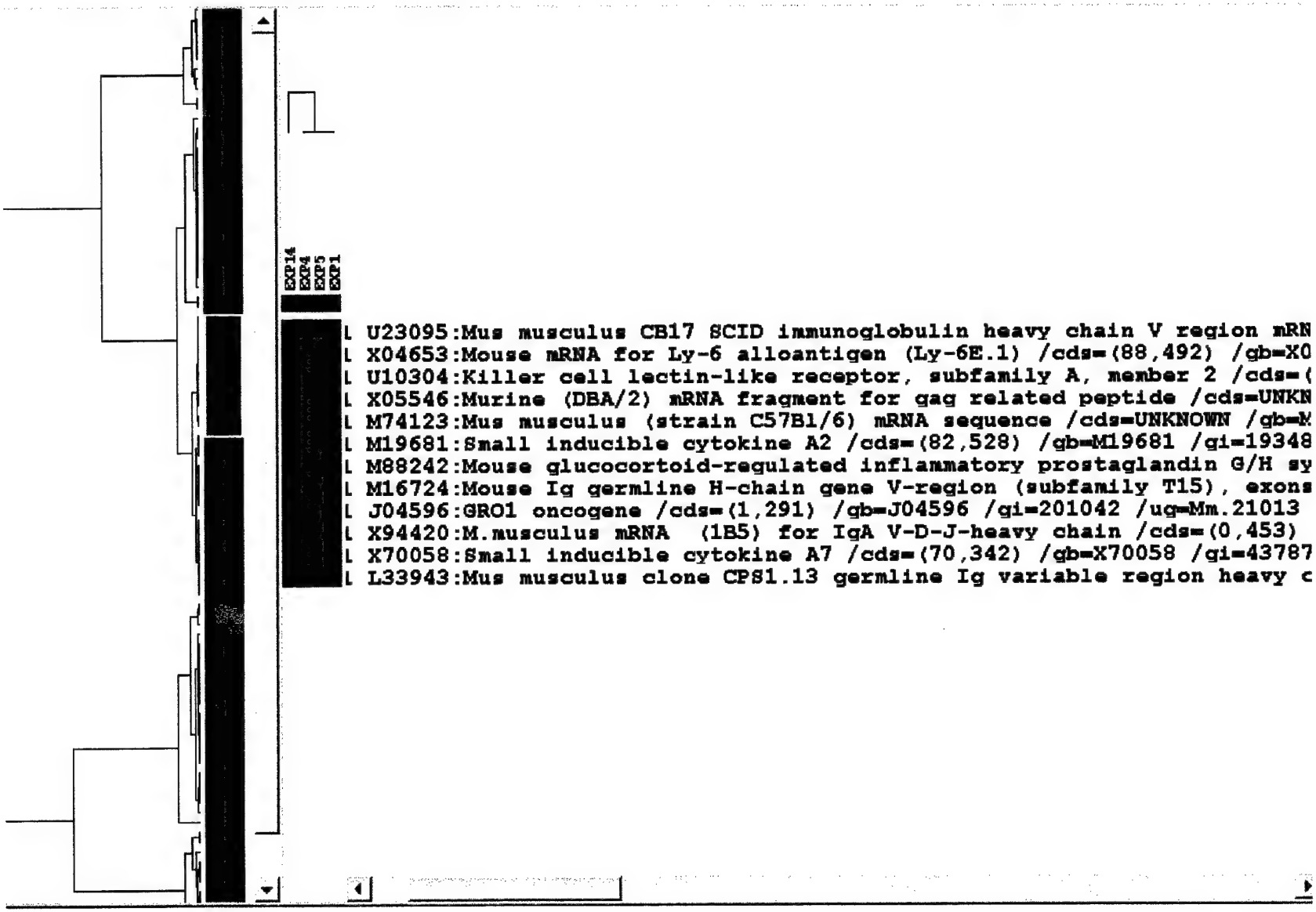
Genes upregulated by LVS and down regulated by LPS



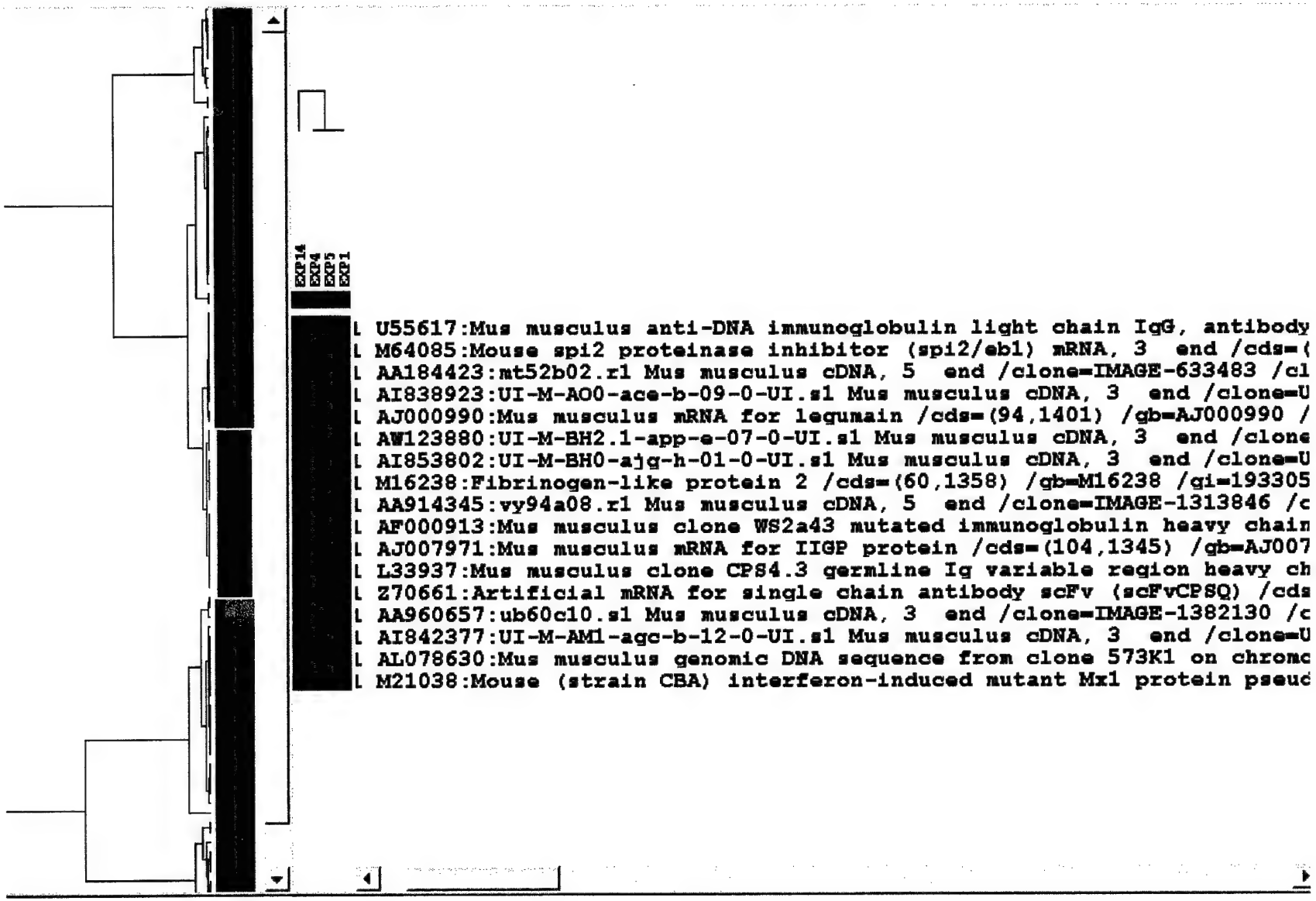
Genes upregulated by LVS and unvhanged by LPS



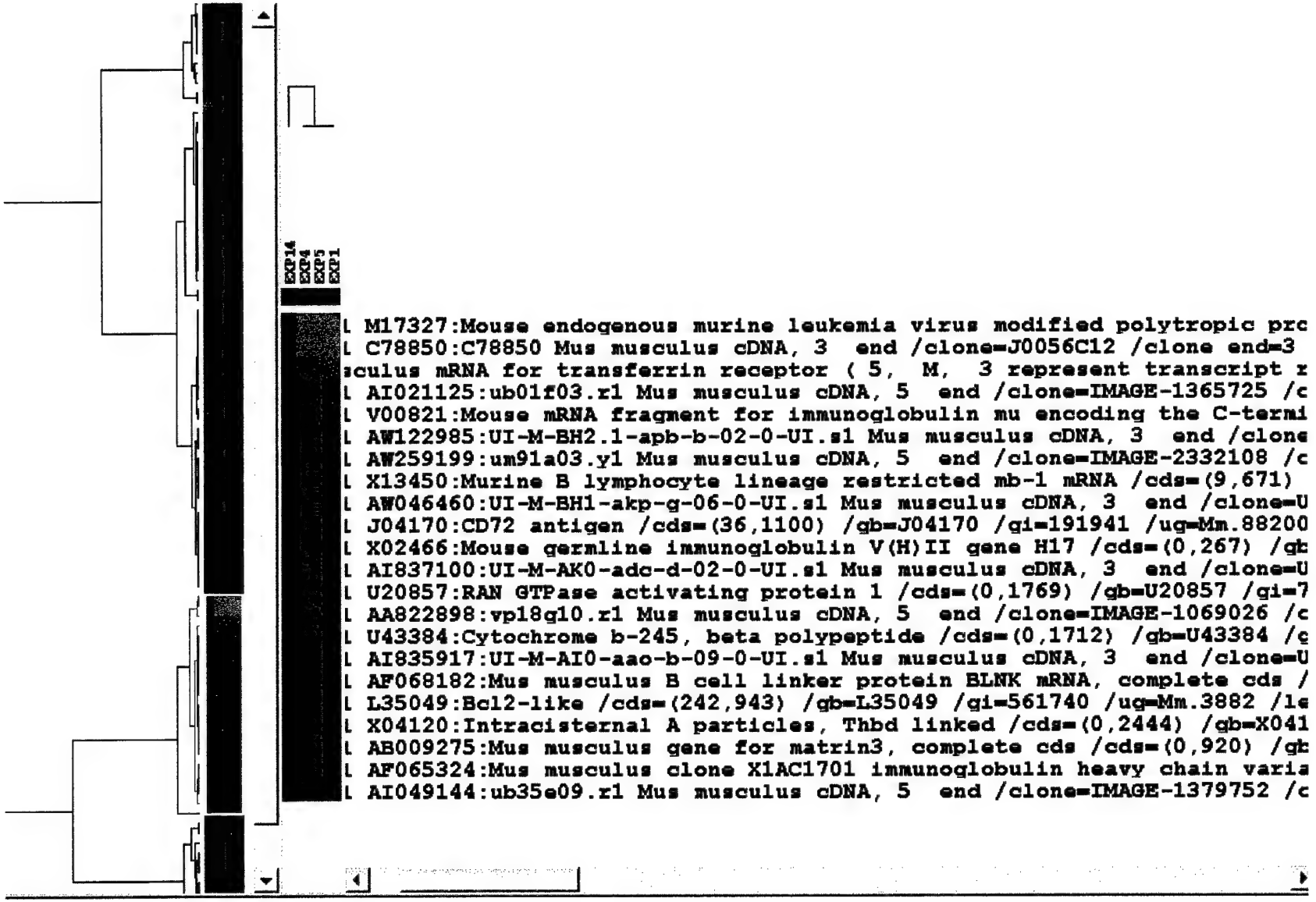
Genes upregulated by LVS and LPS



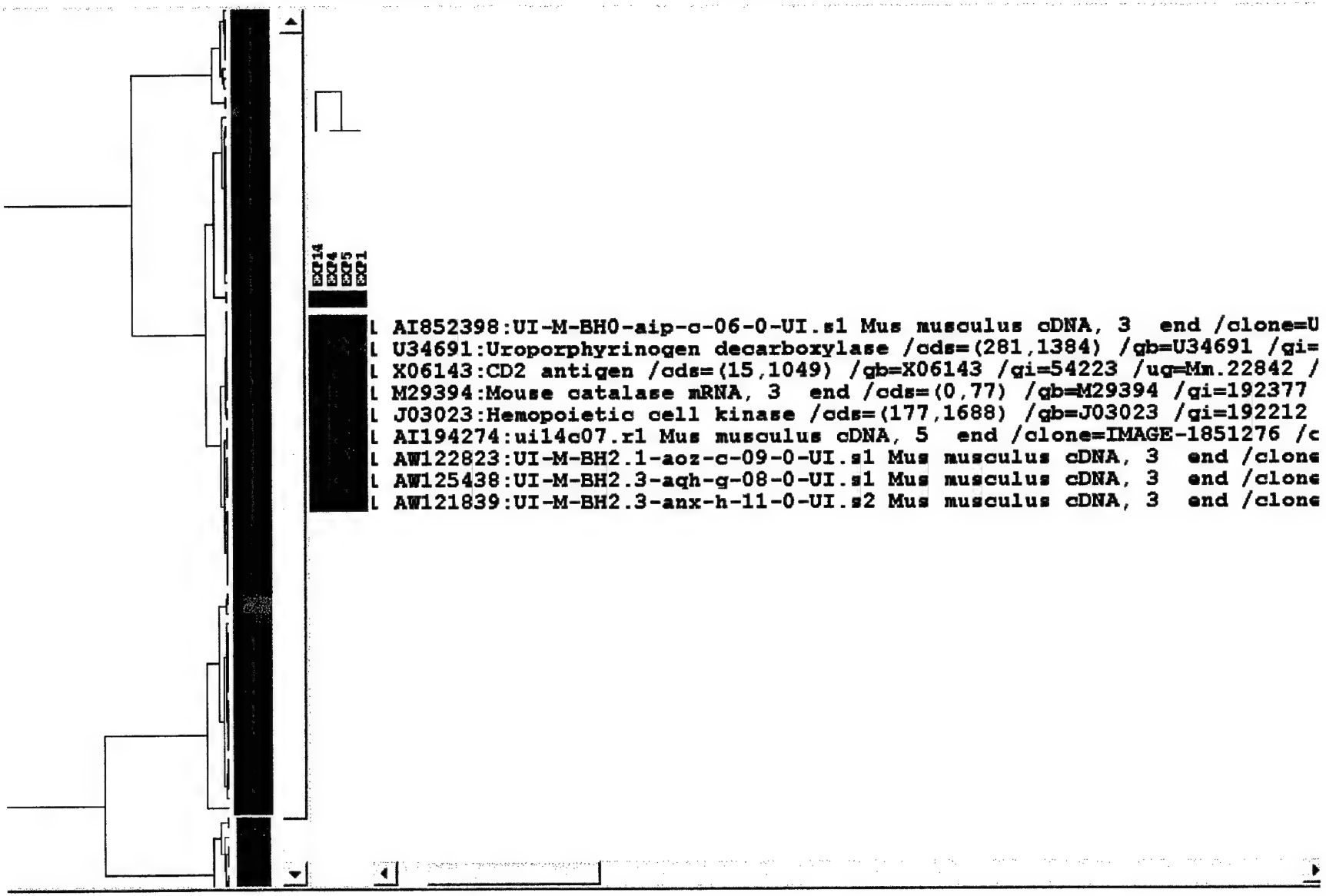
Highly Expressed Genes upregulated slightly by LVS and LPS

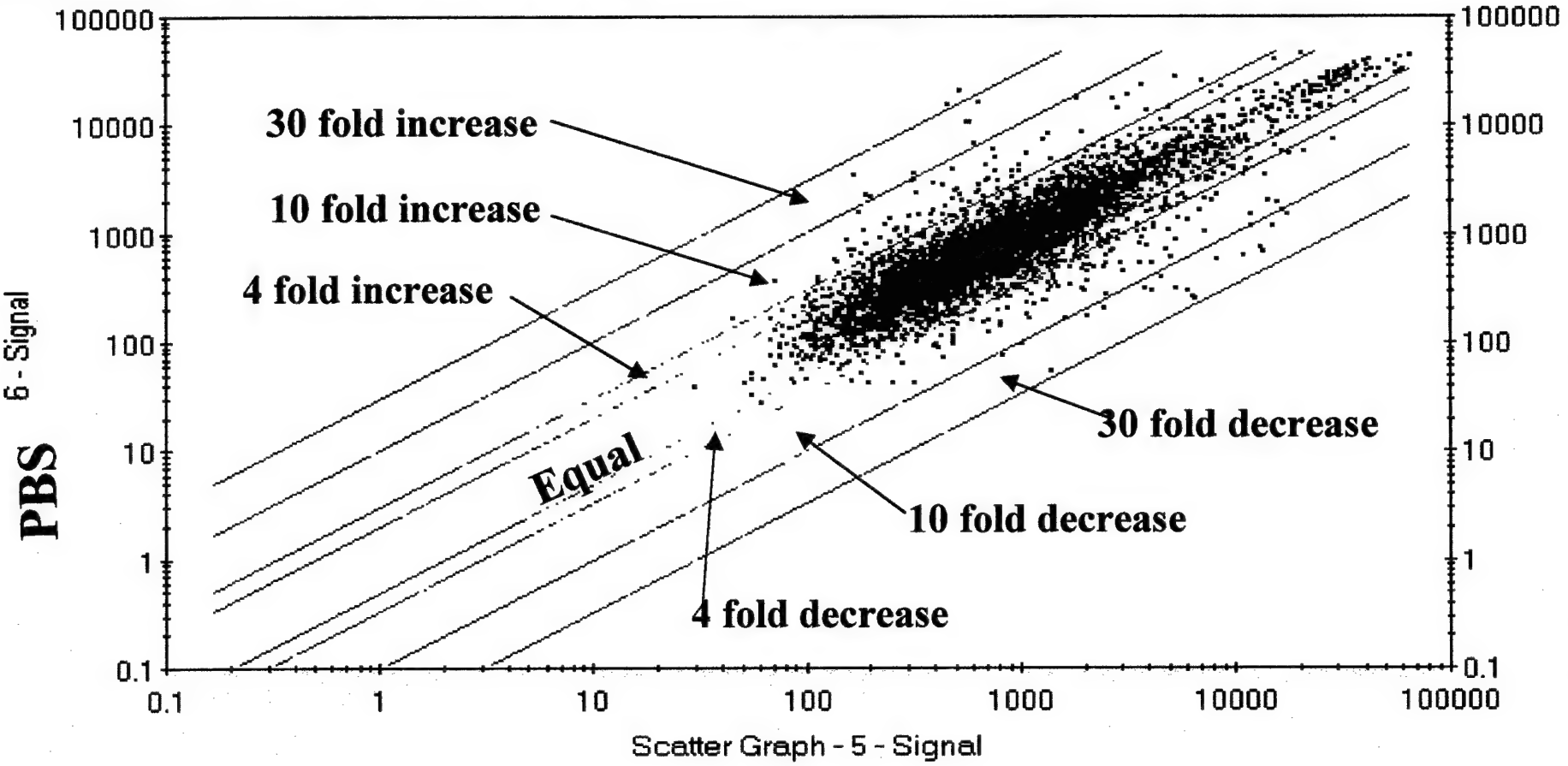


Genes down regulated by LVS and up regulated by LPS

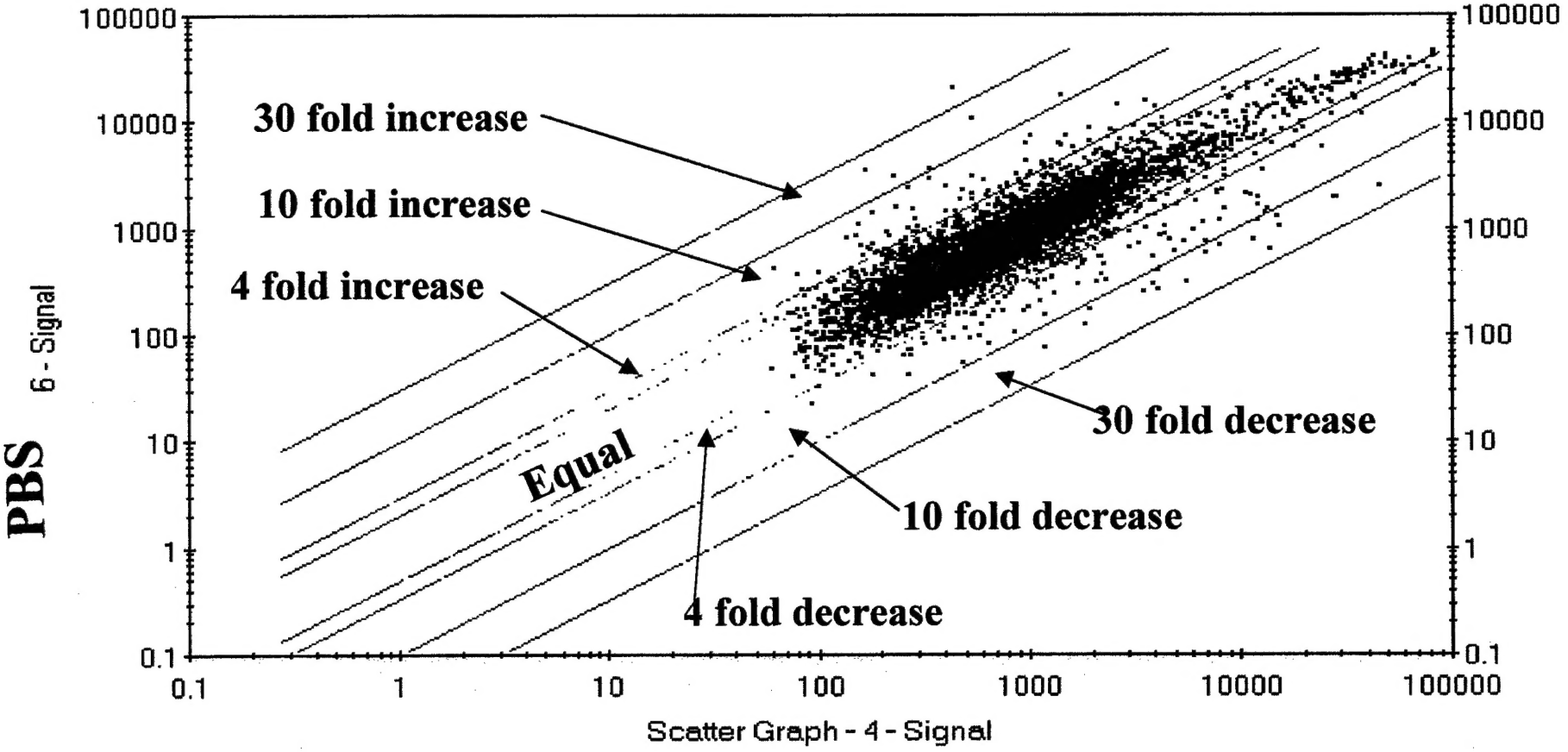


Genes downregulated by LVS and LPS





Tularensis ip



Tularensis id (not fatal)

AJ292757	TUBB1 gene for human beta tubulin 1, cl	3.692	2.431
NM_006366	adenylyl cyclase-associated protein 2 (C	1.115	0.557
NM_007281			
AF146798	clone IMAGE:120153 mRNA sequence	0.911	0.614
U00959	Human clone KDB2.12 (CAC)n/(GTG)n repeat	1.547	1.556
AK021666	cDNA FLJ11604 fis, clone HEMBA1003937	1.013	5.931
NM_004796			
AK021784	cDNA FLJ11722 fis, clone HEMBA1005311	1.892	1.029
NM_006338	glioma amplified on chromosome 1 protei	1.175	2.89
AJ011597	trapped 3' terminal exon, clone B2F11	0.99	1.585
NM_005990	serine/threonine kinase 10 (STK10) mRNA	1.279	2.572
NM_015599	N-acetylglucosamine-phosphate mutase; D	0.323	0.52
NM_020361	carboxypeptidase B precursor (CPAH), mR	1.495	0.695
X05826			
NM_014229	solute carrier family 6 (neurotransmitt	1.147	0.257
NM_017698	hypothetical protein FLJ20173 (FLJ20173	1.87	1.153
AL122039	mRNA; cDNA DKFZp434E0572 (from clone DKF	0.816	1.44
AB037802	mRNA for KIAA1381 protein, partial cds	4.292	2.434
NM_018612	hypothetical protein PRO1995 (PRO1995),	1.147	1.147
AF252280	KLHL1 antisense RNA, partial sequence	0.601	0.666
AK021749	cDNA FLJ11687 fis, clone HEMBA1004960	1.576	1.22
X98415	H.sapiens hypoxically inducible mRNA seq	0.613	1.6
NM_002844	protein tyrosine phosphatase, receptor	1.08	0.981
NM_000352	ATP-binding cassette, sub-family C (CFT	0.691	0.379
AK021971	cDNA FLJ11909 fis, clone HEMBB1000099	0.525	1.029
NM_014677	KIAA0751 gene product (KIAA0751), mRNA	1.798	0.587
NM_004482	UDP-N-acetyl-alpha-D-galactosamine:poly	1.115	0.795
NM_016248	A-kinase anchoring protein 220 (LOC5170	2.649	1.368
AL080106	mRNA; cDNA DKFZp566O053 (from clone DKFZ	1.367	1.224
NM_017414	ubiquitin specific protease 18 (USP18),	0.658	0.981
D28381	Human mRNA for osteonectin, 5'UTR (seque	1.299	0.952
AF085975	full length insert cDNA clone YT86G11	0.474	3.566
AK002211	cDNA FLJ11349 fis, clone PLACE4000650,	2.131	1.628
AL389981	mRNA full length insert cDNA clone EUROI	2.128	3.326
NM_006502	polymerase (DNA directed), eta (POLH) m	1.95	1.944
AF070578	clone 24674 mRNA sequence	0.864	1.415
AL137645	mRNA; cDNA DKFZp586D0924 (from clone DKF	1.165	2.035
AL137363	mRNA; cDNA DKFZp434D1026 (from clone DKF	1.18	1.055
NM_006373	membrane protein of cholinergic synapti	2.292	2.001
AL389983	mRNA full length insert cDNA clone EUROI	1.023	1.13
NM_005059	relaxin 2 (H2) (RLN2) mRNA	1.428	1.234
AK022120	cDNA FLJ12058 fis, clone HEMBB1002092	1.386	0.943
AL157691	mRNA; cDNA DKFZp586J2324 (from clone DKF	2.01	1.525
NM_000435	Notch (Drosophila) homolog 3 (NOTCH3) mR	1.911	0.622
AK000772	cDNA FLJ20765 fis, clone COL08282, high	1.651	1.605
AK026523	cDNA: FLJ22870 fis, clone KAT02506, hig	2.088	1.133

[redacted]

		[redacted]		
NM_018459	uncharacterized bone marrow protein BM0	314.41	87.571	281.697
NM_004591	small inducible cytokine subfamily A (C	48.654	55.54	83.767
AB041269	mRNA for keratin 19, partial cds, isola	8.329	8.613	68.9
NM_002090	GRO3 oncogene (GRO3) mRNA	69.177	48.69	43.453
NM_000675	adenosine A2a receptor (ADORA2A) mRNA	7.765	5.536	40.996
NM_012099	CD3-epsilon-associated protein; antisen	31.258	26.795	35.349
M28983	[redacted]	34.188	57.857	34.827
NM_006890	carcinoembryonic antigen-related cell a	4.679	4.262	29.131
NM_003051	solute carrier family 16 (monocarboxyli	3.556	2.053	26.823
M55646	[redacted]	4.444	6.214	24.077
AK022746	cDNA FLJ12684 fis, clone NT2RM4002460,	2.36	2.085	21.8
NM_018643	triggering receptor expressed on myeloi	7.425	7.407	21.134
NM_016327	beta-ureidopropionase (LOC51733), mRNA	2.698	3.614	20.623
NM_002309	leukemia inhibitory factor (cholinergic	16.792	11.865	20.331
NM_000267	neurofibromin 1 (neurofibromatosis, von	2.267	2.16	20.093
AB032945	mRNA for KIAA1119 protein, partial cds	2.059	2.131	19.484
NM_018593	hypothetical protein PRO0813 (PRO0813),	21.932	15.996	18.07
AK026853	cDNA: FLJ23200 fis, clone KAIA38871	3.765	2.195	18
AF035035	clone S12G10K myosin-reactive immunoglo	2.923	2.403	17.668
AK025784	cDNA: FLJ22131 fis, clone HEP20245	6.29	8.109	16.873
NM_020370	inflammation-related G protein-coupled	3.066	5.045	16.737
NM_002089	GRO2 oncogene (GRO2) mRNA	18.556	24.314	16.69
NM_016579	8D6 antigen (LOC51293), mRNA	8.343	4.721	16.456
NM_013371	[redacted]	7.858	3.491	15.913
NM_015515	DKFZP434G032 protein (DKFZP434G032), mR	2.423	2.308	14.899
M15330	[redacted]	[redacted]	[redacted]	[redacted]
AL389942	mRNA full length insert cDNA clone EUROI	6.298	11.447	14.289
NM_001364	discs, large (Drosophila) homolog 2 (ch	2.187	2.971	14.094
AF116652	PRO0813 mRNA, complete cds	5.23	8.098	13.89
NM_016224	SH3 and PX domain-containing protein SH	10.256	11.007	13.713
NM_014769	KIAA0087 gene product (KIAA0087), mRNA	3.367	2.786	13.619
NM_004633	interleukin 1 receptor, type II (IL1R2)	3.755	4.71	13.595
NM_012285	potassium voltage-gated channel, subfam	3.744	6.933	12.884
M21551	Human neuromedin B mRNA, complete cds	8.045	10.051	12.477
NM_000417	interleukin 2 receptor, alpha (IL2RA) m	4.543	3.797	12.416
NM_001218	carbonic anhydrase XII (CA12), mRNA	7.916	8.724	12.257
NM_004484	glypican 3 (GPC3) mRNA	2.153	5.367	11.695
NM_000582	secreted phosphoprotein 1 (osteopontin,	6.676	8.313	11.6
NM_014809	KIAA0319 gene product (KIAA0319), mRNA	2.227	5.465	11.6
NM_002343	lactotransferrin (LTF) mRNA	2.385	2.162	11.598
NM_007351	multimerin (MMRN), mRNA	2.223	3.7	11.42
AB002344	Human mRNA for KIAA0346 gene, partial cd	2.505	2.856	11.026
X98266	H.sapiens mRNA for ligase like protein,	7.7	2.629	11

Blue section
Upregulated in MDR 3hrs only

		24hrs Ra vs MDR	24hrs uninf vs MDR	24hrs Ra vs MDR monocyte
NM_004260	RecQ protein 4 (RECQ4) mRNA	1.795	1.267	0.744
AK023022	cDNA FLJ12960 fis, clone NT2RP2005605,	0.714	1.379	1.136
K03191	Human cytochrome P-1-450 (TCDD-inducible	0.768	0.917	0.36
AK026196	cDNA: FLJ22543 fis, clone HSI00212	1.835	1.032	1.888
AL133077	mRNA; cDNA DKFZp434O0117 (from clone DKF	0.01*	2.315	0.01*
NM_004700	potassium voltage-gated channel, KQT-li	1.549	0.451	1.892
AL050183	mRNA; cDNA DKFZp586A2223 (from clone DKF	1.504	7.089	1.259
NM_002457	mucin 2, intestinal/tracheal (MUC2) mRN	1.39	1.385	1.658
AF161453	HSPC335 mRNA, partial cds	1.366	3.207	1.882
NM_007341	SH3-binding domain glutamic acid-rich p	1.112	1.275	0.724
NM_016591	core 2 beta-1,6-N-acetylglucosaminyltra	0.586	4.964	0.721
AK024244	cDNA FLJ14182 fis, clone NT2RP2004675	7.77	1.28	5.385
NM_003112	Sp4 transcription factor (SP4) mRNA	0.336	5.506	NO DATA
AL162056	mRNA; cDNA DKFZp761N1024 (from clone DKF	1.923	3.46	1.383
AL080194	mRNA; cDNA DKFZp434B192 (from clone DKFZ	1.692	1.05	1.457
NM_000589	interleukin 4 (IL4), mRNA	1.053	2.208	2.472
NM_000827	glutamate receptor, ionotropic, AMPA 1	2.08	0.983	2.543
NM_002236	potassium voltage-gated channel, subfam	1.902	1.659	1.777
NM_006914	RAR-related orphan receptor B (RORB), m	0.673	2.38	2.068
NM_006299	zinc finger protein 193 (ZNF193) mRNA	1.405	1.391	9.571
NM_004076	crystallin, beta B3 (CRYBB3), mRNA	2.442	0.557	1.914
NM_004155	protease inhibitor 9 (ovalbumin type) (P	5.055	0.682	11.497
AL137555	mRNA; cDNA DKFZp434H0820 (from clone DKF	3.483	1.893	4.662
NM_002153	hydroxysteroid (17-beta) dehydrogenase	0.454	1.894	1.252
D87445	mRNA for KIAA0256 protein, partial cds	1.615	1.3	3.125
NM_014467	sushi-repeat protein (SRPUL), mRNA	1.181	3.869	0.894
AF039697	antigen NY-CO-31 (NY-CO-31) mRNA, parti	1.27	0.982	1.027
AF086129	full length insert cDNA clone ZA83B04	1.798	1.816	2.145
NM_017699	hypothetical protein FLJ20174 (FLJ20174	1.652	1.977	2.426
NM_003679	kynurenine 3-monooxygenase (kynurenine	1.509	2.539	3.692
U92978	clone DT1P1A2 mRNA, CAG repeat region	1.334	1.511	1.8
NM_004575	POU domain, class 4, transcription fact	1.616	1.02	14.3
AK026912	cDNA: FLJ23259 fis, clone COL05779	0.636	0.449	3.407
AF029777	histone acetyltransferase (GCN5) mRNA,	1.931	2.271	1.344
NM_015888	hook1 protein (HOOK1), mRNA	1.514	2.49	2.486
NM_001911	cathepsin G (CTSG) mRNA	1.717	3.738	1.972
NM_003270	transmembrane 4 superfamily member 6 (T	1.089	2.577	1.139
NM_001513	glutathione transferase Zeta 1 (GSTZ1) m	2.59	2.298	1.978
U79273	Human clone 23933 mRNA sequence	4.681	0.715	4.375
Z36784	H.sapiens (xs128) mRNA, 380bp	1.638	2.412	8.399
AF134401	putative espin mRNA, partial cds	1.497	3.949	4.57
NM_014662	KIAA0645 gene product (KIAA0645), mRNA	1.253	31	3.284
NM_006637	olfactory receptor, family 5, subfamily	1.656	1.096	1.78